

DEPARTMENT OF HEALTH AND HUMAN SERVICES

NOTE TO THE FILE (BNF0035)

September 6, 1996

Subject: Roundup Ready (Glyphosate Tolerant) Corn & Insect Protected/Roundup Ready Corn

Keywords:

Corn, insect resistant, *Bacillus thuringiensis* subsp. *kurstaki*, *cryIA(b)*, Insect control protein CryIA(b), *Ostrinia nubilalis*, European Corn Borer, *hsp70* intron, Glyphosate herbicide tolerant (Roundup Ready), CP4 EPSPS, 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS protein), *gox*, glyphosate oxidoreductase (GOX protein), *nptII*, neomycin phosphotransferase II (NPTII protein).

Background

In a submission dated July 2, 1996, Monsanto company provided summary information to support their safety and nutritional assessment of new corn lines MON 802, 805, 830, 831, and 832.

Intended Effect and Food/Feed Use

The intended technical effects of these genetic modifications of corn are i) to confer resistance to the feeding of the European Corn Borer (ECB), *Ostrinia nubilalis*, a lepidopteran pest insect and/or ii) to confer tolerance to glyphosate, the active ingredient in the herbicide Roundup® (hence, Roundup Ready™). Monsanto states that insect resistance is conferred by the insecticidal protein cryIA(b), which is reported to be identical to that found in nature and in commercial insecticidal formulations obtained from *Bacillus thuringiensis*. Corn lines so modified, MON 802 and 805, also resist the feeding of another lepidopteran pest insect, the southwestern corn borer. The firm indicates that one protein, 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS), conferring tolerance to the herbicide glyphosate, is equivalent to that present in modified soybeans and cotton, about which Monsanto has previously informed the Agency. The GOX protein also confers glyphosate tolerance. Corn lines MON 830, 831, and 832 were modified to express only the herbicide tolerance trait (Roundup Ready: RR). Corn lines MON 802 and 805 are both insect resistant and Roundup Ready (IR/RR).

The firm reports that corn grain and its derivatives are used in many human foods, but 50 to 60% of the grain produced annually in the United States is used for animal feed. Human food products include starch, high fructose corn syrup, ethanol, and corn oil. Whole plant corn silage, which makes up 10 to 12% of annual corn acreage, is a major ruminant feedstuff.

Molecular Alterations and Characterization

The novel genetic material contained in lines MON 802, 805, 830, 831, and 832 was inserted into the corn genotype, Hi-II, using particle acceleration transformation. The transformation vectors included several plasmids containing one or more of the following genes: *cryIA(b)*, *CP4 EPSPS*, and *gox*. *CryIA(b)* confers insect resistance, while the *CP4 EPSPS* and *gox* genes confer glyphosate tolerance. Herbicide tolerance was both a selection aid to identify modified plant cells and a desired commercial trait. Additionally, a *nptII* gene, encoding the enzyme neomycin phosphotransferase II (NPTII), was inserted under the control of its own bacterial promoter, enabling plasmid manipulation in bacterial systems.

Monsanto states that the *cryIA(b)* gene used to produce MON 802 and 805 IR/RR corn lines is a modification of the *cryIA(b)* gene from *Bacillus thuringiensis* subsp. *kurstaki* strain HD-1. For these lines and RR corn lines 830, 831, and 832, the *CP4 EPSPS* gene was derived from *Agrobacterium* sp. strain CP4, and the *gox* gene was cloned from *Ochrobactrum anthropi* (formerly *Achromobacter*) strain LBAA. All genes, except *nptII*, are reported to be under the control of the E35S promoter and the corn *hsp70* intron. In addition, these genes contain the NOS 3' termination sequence.

The specific transformation vectors used were PV-ZMGT03 (*gox*) and PV-ZMBK15 (*CP4 EPSPS*, *cryIA(b)*) for MON 802 (IR/RR), PV-ZMBK10 (*gox*, *CP4 EPSPS*, *cryIA(b)*) for MON 805 (IR/RR), and PV-ZMBK07 (*cryIA(b)*) and PV-ZMGT10 (*CP4 EPSPS*, *gox*) for MON 830, 831, and 832 (RR). All plasmids contained the *nptII* gene under the control of a bacterial promoter and an origin of replication (*ori*) from pUC, according to Monsanto.

To exhibit herbicide tolerance, the CP4 EPSPS protein must be translocated into the chloroplast. To achieve this, the chloroplast transit peptide coding sequence (CTP2) from the *Arabidopsis thaliana* EPSPS was fused to the N-terminus of the CP4 EPSPS protein. The transit peptide is cleaved from the enzyme after arrival in the chloroplast. The GOX enzyme also requires translocation into the chloroplast for activity. Translocation is accomplished by use of another transit peptide, CTP1, derived from the ribulose-1,5-bisphosphate carboxylase (SSUIA) gene from *A. thaliana*. The *hsp70* intron was used to increase transcription levels of both herbicide tolerance genes.

Monsanto determined the insert number and the genetic elements present in each insert by appropriate restriction enzyme digestion followed by Southern blot analyses. MON 802 contains two closely linked inserts: a 23 kilobase (Kb) insert with *cryIA(b)*, *CP4 EPSPS*, *gox*, *nptII*, and *ori*-pUC; and a 8 Kb insert with *gox*, *nptII*, and *ori*-pUC. MON 805 contains a single 17 Kb insert with *cryIA(b)*, *CP4 EPSPS*, *gox*, and *nptII*/ori-pUC. MON 830 has one 14 Kb insert containing *CP4 EPSPS*, *gox*, and the *nptII*/ori-pUC backbone. MON 831 has one 13.5 Kb insert containing *CP4 EPSPS*, *gox* and *nptII*/ori-pUC. MON

832 has one 16 Kb insert containing *CP4 EPSPS*, up to three copies of *gox*, and two *nptII/ori-pUC* sequences (one complete and one rearranged). Monsanto reports that segregation data obtained for three generations indicated stable introduction at a single site with Mendelian segregation for MON 805, 830, 831, and 832. The two inserts present in MON 802 are reported to be closely linked, since they segregated as a single locus through three generations. The firm indicates that the inserts were stable through at least four generations.

#### Expressed Proteins/ Regulatory Considerations

Monsanto states that the *cryIA(b)* protein present in MON 802 and 805 is identical to that present in nature and commercial microbial preparations approved by the Environmental Protection Agency (EPA). The safe use of insecticidal proteins as pesticides and the use of selectable markers as pesticidal inert ingredients in the development of insect resistant plant varieties are regulated by EPA under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug, and Cosmetic Act (FFDCA). Therefore, we have not addressed the safe use of *cryIA(b)* as a pesticide or the safe use of *CP4 EPSPS* and *GOX* as pesticidal inert ingredients.

Monsanto examined the expression levels of introduced proteins in leaf, whole plant tissue, and grain and reported that levels were low. The firm states that since the *nptII* gene is under control of a bacterial promoter, expression would not be expected in corn.

#### Nutritional Assessment

##### GRAIN

Compositional analyses by Monsanto for grain from the new corn varieties included proximate analyses (protein, fat, ash, and moisture), amino acid composition, fatty acid profile, calcium and phosphorus. Fiber content was determined using the crude fiber analysis for MON 802 and 805, while acid detergent fiber (ADF) and neutral detergent fiber (NDF) methodologies were utilized for varieties 830, 831, and 832. Values obtained for the new corn lines were comparable to appropriate control lines and to published literature values for corn varieties currently on the market.

##### VEGETATIVE TISSUES

Compositional analyses by Monsanto for forage from the new corn lines included proximate analyses (protein, fat, ash, carbohydrates, and moisture), ADF, and NDF. Values obtained for the new corn lines were comparable to appropriate controls.

Conclusions

Monsanto has concluded that "Corn modified to be resistant to lepidopteran insect pests and/or tolerant to Roundup herbicide is not materially different in composition, safety, or any relevant parameter from corn now grown, marketed, and consumed." At this time, the Agency considers the consultation on corn lines containing transformation events MON 802, 805, 830, 831, and 832 to be complete.

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